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Variant hemoglobin phenotypes may account for differential erythropoiesis-stimulating agent dosing in African-American hemodialysis patients

Vimal K. Derebail[1,2], Patrick H. Nachman[1], Nigel S. Key[3], Heather Ansede[4], Ronald J. Falk[1], Wayne D. Rosamond[2] and Abhijit V. Kshirsagar[1]

[1]Division of Nephrology and Hypertension, Department of Medicine, UNC Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [2]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [3]Division of Hematology and Oncology, and Program in Hemostasis and Thrombosis, Department of Medicine, Carolina Cardiovascular Biology Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA and [4]Renal Research Institute, New York, New York, USA

African-American patients with end-stage renal disease have historically lower hemoglobin concentrations and higher requirements of erythropoiesis-stimulating agent (ESA). While disparities in health-care access may partially explain these findings, the role of variant hemoglobin, such as sickle trait, has not been investigated. To clarify this, we evaluated 154 African-American patients receiving in-center hemodialysis with available hemoglobin phenotyping. The primary exposure was any abnormal hemoglobin variant and the primary outcome of higher-dose ESA was defined as a dose of 6500 or more units per treatment. Logistic regression assessed the association between variant hemoglobin and higher-dose ESA. Covariates included age, gender, diabetes, iron parameters, intravenous iron dose, parathyroid hormone, albumin, phosphorus, body mass index, vascular access type, hospitalization/missed treatments, smoking status, alcohol abuse, and gastrointestinal bleeding. Of 33 patients with variant hemoglobin, 24 had HbAS and 9 had HbAC. Univariate odds of higher-dose ESA among those with hemoglobin variants were twice that of those with the normal HbAA phenotype (odds ratio 2.05). In multivariate models, the likelihood of higher-dose ESA had an odds ratio of 3.31 and the nature of this relationship did not change in Poisson regression or sensitivity analyses. Hence, our findings may explain, in part, the difference in ESA dosing between Caucasians and African-Americans with end-stage renal disease but await further study.

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Correspondence: *Vimal K. Derebail, Division of Nephrology and Hypertension, Department of Medicine, UNC Kidney Center, University of North Carolina at Chapel Hill, CB # 7155, Chapel Hill, North Carolina 27599, USA. E-mail: vimal_derebail@med.unc.edu*

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Historically, African-American patients with end-stage renal disease (ESRD) have had lower hemoglobin concentrations at the start of dialysis when compared with other racial or ethnic groups.[1] The difference in hemoglobin persists into the first year of renal replacement therapy despite similar treatment protocols.[1] Furthermore, observational data show that African-American patients receiving chronic hemodialysis ($\geqslant$90 days) require larger doses of erythropoiesis-stimulating agents (ESAs) to achieve a similar level of hemoglobin after adjustment for other modifying factors.[2] African-Americans receive $\sim$12% more ESA than white patients despite lower rates of hospitalization and catheter use.[2] Proposed explanations for this observation have focused on disparities in access to predialysis care among African-Americans,[2] although these seem insufficient to explain the persistence of higher ESA requirements throughout dialysis care. An alternative or additional possibility is that lower hemoglobin levels and higher ESA dosing may be reflective of an underlying hemoglobinopathy.[2] Further, potential modifiers to ESA therapy may have relevance to the planned bundling of ESRD payment that presently does not include race/ethnicity nor hemoglobin variants in its case-mix adjustment.[3]

Recently, we reported a high prevalence of heterozygosity for hemoglobin S (HbAS) and hemoglobin C (HbAC) in a group of African-Americans with ESRD receiving renal replacement therapy.[4] Although typically described as a benign carrier state, individuals with either HbAS or HbAC may experience clinical complications due to changes to red-cell rigidity under situations of stress[5–9] that could lead to low-grade sickling and hemolysis. Speculatively, such changes could occur during the hemodialysis procedure.

We therefore postulated that patients with these hemoglobinopathies receiving hemodialysis would require higher doses of ESAs. We examined the association of these variant hemoglobins with ESA dosing in a group of

African-American patients receiving regular outpatient in-center hemodialysis.

RESULTS

Of the 154 patients included in our analysis with at least 20 treatments over the 3-month study period, 33 had abnormal hemoglobin phenotypes. Of these, 24 had sickle-cell trait or HbAS and 9 had HbAC trait. Patients among all hemoglobin phenotypes were similar in age (Table 1). Sex was distributed similarly in all groups. Mean achieved hemoglobin was also similar between those with normal and variant hemoglobin phenotype. Patients with variant hemoglobin had received a larger median dose of erythropoietin, particularly those with HbAC trait—although not statistically significant; however, iron dosing per treatment was similar. Median dialysis vintage was notably greater in patients with hemoglobin variants ($P = 0.007$). The average number of treatments over the 3-month period was not significantly different among all groups, although those with the normal hemoglobin phenotype had a tendency to miss more treatments.

No statistically significant differences were noted in adequacy (Kt/*V*), serum albumin, or iron saturation (Table 1). Ferritin was not different when all hemoglobin variants were viewed collectively, but did appear to be somewhat greater among HbAS patients and slightly less among HbAC patients. Although patients with variant hemoglobin tended to have higher intact parathyroid hormone levels, largely driven by the HbAS patients, these differences were not statistically significant.

The frequencies of alcohol abuse, smoking, and history of gastrointestinal bleeding (GI bleed) were similar between patients with normal and variant hemoglobin. About two-thirds of all patients were diabetic.

From our population of potential African-American hemodialysis patients, 17 patients with <20 treatments were not included, all of whom had normal hemoglobin phenotypes. Five received no treatments over the study period and had received a transplant, transferred, or expired. The remaining 12 patients were predominantly male (10 of 12) and somewhat older (mean age 68.4 years) than the included normal hemoglobin phenotype population. These excluded patients had median ESA dose of 4937.43 U/treatment (interquartile range (IQR) 3107.1, 16062.5) and lower average hemoglobin (10.4 mg/dl, s.d. 1.6 mg/dl).

Table 1 | Baseline characteristics of study population by hemoglobin phenotype

	Normal hemoglobin (HbAA; *n*=121)	Any variant hemoglobin[a] (*n*=33)	Sickle cell trait (HbAS; *n*=24)	Hemoglobin C trait (HbAC; *n*=9)
Age (years)	59.5 (13.9)	59.6 (13.8)	59.4 (12.9)	60.1 (16.8)
Sex				
Male	61 (50.4%)	17 (51.5%)	13 (54.2%)	4 (44.4%)
Female	60 (49.6%)	16 (48.5%)	11 (45.8%)	5 (55.6%)
Body mass index (kg/m^2)[b]	27.7 (23.4, 33.2)	27.1 (23.3, 31.8)	26.1 (23.1, 30.7)	28.9 (24.0, 33.2)
Hemoglobin (mg/dl)	11.8 (0.9)	12.0 (1.0)	12.0 (0.9)	12.2 (1.4)
Erythropoietin dose/treatment (units)[b]	3684.2 (1955, 6756.4)	4897.4 (2425, 8230.8)	4475.6 (2232.5, 8092)	7092.1 (2980, 8230.8)
Intravenous iron dose/treatment (mg)[b]	12.5 (0, 19.1)	12.8 (0, 16.0)	12.5 (0, 15.9)	12.9 (12.1,17.5)
Interdialytic weight gain (kg)[b]	2.60 (1.90, 3.20)	2.80 (2.30, 3.70)	2.55 (2.30, 3.45)	3.50 (2.40, 4.40)
Number of treatments[b]	39 (37, 40)	39 (38, 40)	39 (38, 40)	38 (32, 40)
Missed treatments (% of prescribed)[b]	0 (0, 5.3)	0 (0, 2.6)	0 (0, 2.5)	0 (0, 5)
Days hospitalized/week[c]	0.17 (0.35)	0.05 (0.17)	0.05 (0.18)	0.08 (0.16)
Dialysis vintage (years)[b]	3.3 (1.7, 6.0)	6.1 (2.5, 8.7)	6.2 (2.9, 9.3)	5.8 (2.1, 6.8)
Kt/*V*	1.52 (0.33)	1.51 (0.27)	1.47 (0.16)	1.64 (0.43)
Albumin (mg/dl)	3.90 (0.32)	3.96 (0.34)	3.96 (0.34)	3.94 (0.36)
Ferritin (ng/ml)	723.5 (273.3)	787.6 (277.3)	834.1 (298.3)	663.3 (167.0)
Iron saturation (%)	32.1 (7.6)	31.8 (8.2)	31.2 (8.9)	33.2 (6.1)
Phosphorus (mg/dl)	5.40 (1.34)	5.36 (1.63)	5.40 (1.62)	5.23 (1.74)
iPTH (pg/ml)[b]	340 (240.8, 547.7)	393.3 (250.8, 622.7)	451.6 (269.3, 714.2)	342.8 (169.0, 475.9)
Vascular access				
Arteriovenous fistula	48 (39.7%)	20 (60.6%)	14 (58.3%)	6 (66.7%)
Arteriovenous graft	44 (36.4%)	8 (24.2%)	5 (20.8%)	3 (33.3%)
Indwelling catheter	39 (24.0%)	5 (15.2%)	5 (20.8%)	0
Diabetes	74 (61.2%)	22 (66.7%)	16 (66.7%)	6 (66.7%)
History of smoking	19 (15.7%)	5 (15.2%)	2 (8.3%)	3 (33.3%)
History of alcohol abuse	11 (9.1%)	2 (6.1%)	2 (8.3%)	0
History of GI bleed	14 (11.6%)	3 (9.1%)	1 (4.2%)	2 (22.2%)

Abbreviations: GI bleed, gastrointestinal bleeding; iPTH, intact parathyroid hormone.
[a]Includes HbAS and HbAC.
[b]Median (IQR).
[c]Week defined as three dialysis treatments (all patients assigned to thrice weekly dialysis).

Data for several covariates were missing in up to 50% of these observations.

Higher-dose ESA

Of the 121 patients with normal hemoglobin, 32 (26.5%) were found to require higher-dose ESA as compared with 14 (42.4%) of the 33 patients with abnormal hemoglobin variants. Patients requiring higher-dose ESA (Table 2) had a lower achieved hemoglobin ($P = 0.0002$), lower albumin ($P = 0.004$), lower iron saturation (0.001), higher iron dose per treatment (0.001), more missed treatments ($P = 0.008$), more hospitalizations ($P = 0.007$), and a higher frequency of history of GI bleed ($P = 0.05$). A trend for more common catheter use was noted among those with higher-dose ESA; however, this was not statistically significant. No other major differences were noted between the two dosing groups.

In univariate analysis, the odds of higher-dose ESA (defined as ESA dose $\geqslant$6500 U/treatment) among those with hemoglobin variants were twice that of those with the normal HbAA phenotype (Table 3). In multivariate models, the association strengthened further, reaching statistical significance. The fully adjusted model incorporated all identified potential confounders (dialysis vintage, percent of missed treatments, dialysis unit, serum albumin, iron saturation, iron dose, vascular access type, history of GI bleed, ferritin, intact parathyroid hormone, iron saturation, and Kt/*V*). Individuals with hemoglobin variants had three times the likelihood of higher-dose ESA than individuals with HbAA (odds ratio (OR) 3.31, 95% CI 1.20–9.11). The simplified model generated from backward elimination and change-in-estimate testing adjusted for missed treatments, dialysis access, and albumin produced similar results with OR 3.02 (95% CI 1.26–7.25).

Poisson regression modeling, a more conservative method of effect-size estimation, still revealed higher-dose ESA to be nearly twice as likely among those with variant hemoglobin than HbAA in fully adjusted and simplified models, with an incident rate ratio of 1.94 (95% CI 1.16–3.25) and 2.03 (95% CI 1.24–3.31), respectively (Table 3).

Sensitivity analyses

Using alternative definitions for higher-dose ESA in the same fully adjusted multivariate logistic regression model, we noted a slightly larger effect size at 7000 U/treatment (Table 4) among those with variant hemoglobin. Although variant hemoglobin was still associated with higher odds of higher-dose ESA at definitions of 8000 U/treatment and greater, our effect estimates were substantially smaller and did not reach statistical significance. In addition, further sensitivity analyses defining higher-dose ESA as a weight-based measure, $\geqslant$100 U/kg/treatment, demonstrated effects similar to that of our per-treatment definition—OR 1.9 (0.81–4.43) in univariate analysis (Table 4). When adjusted as in our fully adjusted model, the association also strengthened (OR 2.67, 95% CI 0.93–7.66), and in our simplified model reached statistical significance (OR = 2.74, 95% CI 1.08–6.91).

We compared the 60 Caucasian patients from the same dialysis units with similar inclusion criteria ($\geqslant$20 treatments over the study period). No difference was discernable in ESA dosing between Caucasian and African-American patients with normal hemoglobin variants—median ESA dose 3959.2

Table 2 | Baseline characteristics of study population by need for higher-dose ESA

	ESA <6500 units/treatment (*n*=108)	ESA ⩾6500 units/treatment (*n*=46)
Abnormal hemoglobin[a]	19 (17.6%)	14 (30.4%)
Age (years)	60.3 (13.2)	57.7 (15.3)
Sex		
Male	55 (50.9%)	23 (50%)
Female	53 (49.1%)	23 (50%)
Body mass index (kg/m^2)[b]	27.9 (24.0, 33.3)	26.0 (22.0, 30.7)
Hemoglobin (mg/dl)	12.1 (0.9)	11.4 (0.9)
Erythropoietin dose/ treatment (units)[b]	2677.9 (1511.3, 4051.9)	10522.6 (8230.8, 14851.4)
Intravenous iron dose/treatment (mg)[b]	9.5 (0, 16.6)	16 .0 (10.6, 23.6)
Interdialytic weight gain (kg)[b]	2.54 (1.90, 3.20)	2.60 (2.20, 3.5)
Number of treatments[b]	39 (38, 40)	38 (28, 39)
Missed treatments (% of prescribed)[b]	0 (0, 2.6)	2.5 (0, 16.2)
Days hospitalized/ week[b,c]	0.09 (0.25)	0.27 (0.44)
Dialysis vintage (years)[b]	3.8 (1.6, 7.0)	4.0 (2.1, 6.1)
Kt/*V*	1.50 (0.28)	1.56 (0.39)
Albumin (mg/dl)	3.96 (0.29)	3.80 (0.38)
Ferritin (ng/ml)	743.7 (274.1)	722 (278.0)
Iron saturation (%)	33.3 (7.4)	29.0 (7.6)
Phosphorus (mg/dl)	5.4 (1.3)	5.3 (1.6)
iPTH (pg/ml)[b]	347.7 (223.1, 547.7)	338.5 (261.0, 580.7)
Vascular access		
Arteriovenous fistula	51 (47.2%)	17 (37.0%)
Arteriovenous graft	38 (35.2%)	14 (30.4%)
Indwelling catheter	19 (17.6%)	15 (32.6%)
Diabetes	68 (63.0%)	28 (60.9%)
History of smoking	16 (14.8%)	8 (17.4%)
History of alcohol abuse	8 (7.4%)	5 (10.9%)
History of GI bleed	8 (7.4%)	9 (19.6%)

Abbreviations: ESA, erythropoiesis-stimulating agent; GI bleed, gastrointestinal bleeding; HbAC, hemoglobin C trait; HbAS, hemoglobin S trait; iPTH, intact parathyroid hormone.
[a]Includes HbAS and HbAC.
[b]Median (IQR).
[c]Week defined as three dialysis treatments (all patients assigned to thrice weekly dialysis).

Table 3 | Association of variant hemoglobin and higher-dose ESA[a]

	Univariate analysis		Multivariate analysis[b]		Simplified analysis[c]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Logistic regression						
Variant Hgb	2.05 (0.92–4.56)	0.08	3.31 (1.20–9.11)	0.02	3.02 (1.26–7.25)	0.01
	IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value
Poisson regression						
Variant Hgb	1.60 (0.98–2.64)	0.06	1.94 (1.16–3.25)	0.01	2.03 (1.24–3.31)	0.005

Abbreviations: CI, confidence interval; ESA, erythropoiesis-stimulating agent; HbAS, hemoglobin S trait; OR, odds ratio.
[a]Hemoglobin variants included sickle cell trait (HbAS) and hemoglobin C trait. Higher-dose erythropoiesis-stimulating agent defined as ⩾6500 U/treatment (⩾19,500 U/week). Poisson regression used to more closely approximate risk ratio as odds ratio as determined by logistic regression likely overstates effect estimate, as outcome was common (29.9%).
[b]Multivariate analysis from fully adjusted model—adjusted for percent of missed treatments, albumin, dialysis access, dialysis vintage, Kt/*V*, ferritin, iron saturation, intact parathyroid hormone, dialysis unit, history of gastrointestinal bleeding, and iron dose per treatment.
[c]Simplified analysis or 'final model' derived from full model with removal of covariates by backward elimination via change in estimate testing—adjusted for percent of missed treatments, albumin, and dialysis access.

Table 4 | Association of variant hemoglobin and alternative definitions for higher-dose ESA

	Variant hemoglobin		
	N	OR (95% CI)	*P*
Definition for higher-dose ESA[a]			
⩾6000 U/treatment	14/33	2.96 (1.09–8.01)	0.03
⩾6500 U/treatment	**14/33**	**3.31 (1.20–9.11)**	**0.02**
⩾7000 U/treatment	13/33	4.15 (1.40–12.33)	0.01
⩾8000 U/treatment	9/33	2.43 (0.74–7.98)	0.1
⩾9000 U/treatment	7/33	1.92 (0.58–6.30)	0.3
⩾10,000 U/treatment	7/33	2.16 (0.65–7.21)	0.2
Weight-based definition for higher-dose ESA			
⩾100 U/kg/treatment[a]	11/33	2.67 (0.93–7.66)	0.07
⩾100 U/kg/treatment[b]	11/33	2.74 (1.08–6.91)	0.03

Abbreviations: CI, confidence interval; ESA, erythropoiesis-stimulating agent; OR, odds ratio.
[a]Multivariate analysis adjusted for percent of missed treatments, albumin, dialysis access, dialysis vintage, Kt/*V*, ferritin, iron saturation, iPTH, dialysis unit, history of gastrointestinal bleeding, and iron dose per treatment.
[b]Adjusted for percent of missed treatments, albumin, and dialysis access.
The bold row represents the primary outcome definition for higher-dose ESA used in the initial analysis.

(IQR 1982.1, 7738.5) and 3684.2 (IQR 1955, 6756.4), respectively. As noted earlier, those African-Americans with abnormal hemoglobin variants did have a higher median ESA dose, 4897.4 (IQR 2425, 8230.8), although this was not statistically significant ($P = 0.6$).

DISCUSSION

We found that variants of hemoglobin were significantly associated with the dosing of ESA in this group of 154 African-American patients receiving hemodialysis. Those individuals with an abnormal hemoglobin phenotype were more likely to receive a higher dose of ESA, suggesting a relative resistance to ESA. Sensitivity analyses, using varying definitions of higher-dose ESA, revealed this association to be robust. Our findings associating variant hemoglobin and higher ESA dose raise questions regarding mechanism and concerns about possible implications.

Several possible explanations exist for our observed association. The mutations leading to hemoglobin S and C both produce a structurally abnormal β-globin chain. Precipitation of these chains occurs when the red blood cell is exposed to conditions of stress ultimately leading to increased rigidity of the cells.[5–9] During a hemodialysis session, red blood cells flow through an extracorporeal circuit that is exposed to environmental stressors (low temperature and low partial pressure of oxygen) potentially leading to increased destruction of red blood cells and a shortened half-life. Although we do not have measures of low-grade red-cell destruction in the current cohort, measurement of indices such as serum bilirubin, lactate dehydrogenase, and haptoglobin could be instructive in future studies.

An alternative mechanism for relative ESA resistance may be chronic inflammation. Patients with sickle-cell disease (HbSS) have demonstrated inflammatory changes including elevations in C-reactive protein and interleukin-6.[10,11] A similar, albeit muted, process could occur in patients with a single allele (or heterozygous sickle hemoglobin, HbAS). Inflammation has been suggested to impair both iron utilization and erythropoiesis.[12–15] Numerous studies have shown in the ESRD population that indices of active inflammation, including C-reactive protein, have been associated with ESA resistance.[12,16,17] The presence of variant hemoglobin would have to induce inflammatory responses beyond those already experienced in hemodialysis. Finally, antibodies to erythropoietin may contribute to an impaired ESA response. Such antibodies have been reported in patients with β-thalassemia and sickle/β(+)-thalassemia,[18] but may not be common among ESRD patients.[19] Future studies should investigate the prevalence of such antibodies in ESRD patients in relation to their hemoglobin phenotype.

Although requiring additional confirmation, the findings of our study may have important implications. Exposure to relatively high doses of ESA in the ESRD population has raised safety concerns. The Normal Hematocrit Cardiac Trial, a landmark study, demonstrated that normalizing hemoglobin in ESRD patients was associated with increasing mortality.[20] Recent studies in patients with CKD also suggested higher cardiovascular events and stroke with higher levels of hemoglobin (and concurrently, higher doses

of ESA).[21–23] A secondary analysis of the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) study suggested that these events occur more frequently in those failing to reach their assigned target hemoglobin and requiring higher doses of ESA.[24] Although our two groups of patients with and without variant hemoglobin achieved similar hemoglobin, among those with higher-dose ESA in either group, the achieved hemoglobin was lower. Future studies should focus on determining the association between mortality and higher-dose ESA and variant hemoglobin, and should attempt to separate the effect, if any, of the underlying hemoglobinopathy. At present, the Kidney Disease Outcome Quality Initiative (KDOQI) recommends a target hemoglobin of 11–12 g/dl among patients receiving dialysis therapy, irrespective of the dose of ESA or intravenous iron.[25] With data arising that have associated morbidity with ESA and higher hemoglobin targets, present practice has shifted to a target range of 10–12 g/dl.[26] Altering the goal hemoglobin or setting a maximum dose of ESA may need to be considered, particularly in populations more likely to require higher-dose ESA, such as ours. The potential for a lower target hemoglobin does have credence given the lower hemoglobin concentration found among the general African-American population compared with Caucasians and among the chronic kidney disease population.[27,28]

In addition, our findings may have some relevance to the proposed bundled-payment system instituted by Centers for Medicare and Medicaid Services, which will include all injectable medications, including ESA.[29,30] In its current iteration, the following patient and facility level characteristics will be used to calculate payments—age, body mass index, body surface area, and size of dialysis organization. Race/ethnicity has not been incorporated as one of the adjustment variables, although models using the planned bundling system predict higher costs for African-American patients, driven largely by higher costs for ESAs.[31,32] Variant hemoglobin, more common in this population, may partly explain the higher ESA requirement. Notably, however, in our study population, no difference was detectable between African-Americans as a whole and Caucasians in median dose of ESA, making it difficult to extrapolate our findings to previous population studies. Our variant hemoglobin African-American group did have higher median ESA than Caucasians, albeit not statistically significant, suggesting the potential role of these variants in the previous differences noted. If the same target hemoglobin is presumed to be appropriate in this population, including race, or more specifically these variants, could provide further information for revision of the bundling model. Alternatively, as above, perhaps race-specific or hemoglobin variant-specific targets may need to be devised.

Finally, although variant hemoglobins are unlikely to affect overall mortality in ESRD, investigation of other morbidities associated with variant hemoglobin may be warranted in the ESRD population. Sickle-cell trait has been demonstrated to be potentially prothrombotic.[33,34] Recent evidence has associated ESAs and venous thromboembolism.[23,35] Such a relationship could theoretically place ESRD patients with sickle-cell trait who are receiving ESAs at an enhanced risk for venous thromboembolism and possibly vascular access thrombosis, particularly in those patients with synthetic arteriovenous grafts.[36]

The results of this study must be interpreted in the context of its limitations. Because of the cross-sectional nature of the study, we could not perform adjustment for varying levels of hemoglobin, ESA, and other covariates that are likely to change frequently. Ideally, a prospective study in a larger population over a substantially longer period of time would allow the use of time-dependent analyses and perhaps even marginal structural models.[37] In addition, our population primarily had either sickle-cell trait or HbAS trait, making the findings less applicable to other hemoglobinopathies. It is noteworthy that some patients with normal hemoglobin may have had a false-negative diagnosis of β-thalassemia trait, based on cutoffs for HbA_2 and HbF (>3.1 and $>2\%$, respectively; Laboratory Corporation of America (LabCorp), Raritan, NJ). However, with an assumption that β-thalassemia trait would also alter ESA response,[38] inclusion of these patients with those who had normal hemoglobin would bias our effect toward the null. We have also not been able to identify the role of α-thalassemia in our population, which has been shown to mitigate some of the effects of sickle-cell trait.[39] However, if the proportion of these variants in our patients is representative of the general African-American ESRD population, our findings certainly carry great relevance as at least 20% of African-American patients could be affected.[4]

Our definition of higher-dose ESA is lower than the typical dose noted in the Normal Hematocrit Cardiac Trial.[20] The chosen cutoff point was based upon both the ESA dose distribution in our population and the increase in mortality noted in USRDS data.[40] Furthermore, two other large studies of patients receiving hemodialysis also noted an increase in mortality associated with ESA doses $\geqslant 20{,}000$ U/week, similar to our definition of higher-dose ESA.[41,42] Our findings must also be interpreted in the context of the proposed reductions to target hemoglobin that have been suggested in the wake of the CHOIR and CREATE studies. As a result, usage of ESAs is likely to be lower overall, and it is not clear whether this would be proportional across all patient populations. The differences we have noted for those with variant hemoglobin may be blunted. Our sensitivity analyses demonstrated an effect even with lower cutoff points for our definition of higher-dose ESA, suggesting that our observations may persist in the face of reduced ESA dosing. Finally, data regarding the details of hospitalizations and history of blood transfusions were not available for inclusion in our analyses. We have been unable to correct more directly for any degree of inflammation, as these markers are not routinely obtained in these dialysis units.

In summary, we report a novel observation that, among African-Americans, the presence of variant hemoglobin

(HbAS and HbAC) increases the likelihood of higher-dose ESA. Patients appear to have more than three times the odds of receiving higher doses of erythropoiesis-stimulating agents in order to maintain hemoglobin levels. Confirmation of our findings is needed to explore whether the presence of HbAS and HbAC affects patient safety and outcomes, as well as practice patterns of dialysis clinics in the upcoming era of bundling. Future studies, furthermore, should aim to determine hemoglobin phenotype before initiation of ESA therapy with prospective determination of administered ESA dose and associated morbidity and mortality.

MATERIALS AND METHODS

Study population

Hemoglobin phenotyping was performed in June 2008 for African-American ESRD patients receiving dialysis in four centers. Ascertainment of race/ethnicity was determined by that recorded in administrative data via the Medicare 2728 form. Of the 206 possible African-American patients identified, 188 completed phenotyping; the others missed their scheduled visits for various reasons including hospitalization, travel, or non-adherence. For the purposes of consistency in management and data collections, we chose to evaluate the population of in-center hemodialysis patients, whose ESA dosing is determined by the same protocol (Supplementary Figure S1 online) in all of the represented dialysis centers, and excluded those receiving peritoneal dialysis ($n = 16$). All patients were prescribed to three times weekly dialysis. Furthermore, we required that patients in the study group received a minimum of 20 (or 50% of maximum) prescribed dialysis sessions over the 3-month period to minimize potential bias that could be introduced by patient illness and/or non-compliance. Only one patient was noted to have β-thalassemia trait and was excluded to prevent identity disclosure; 154 patients remained and comprised the final study group. Using a cross-sectional approach, we reviewed information over a 3-month period to minimize variability in laboratory measurements and medication dosing. We also evaluated characteristics of the 17 hemodialysis patients with <20 treatments, who were not included, to ensure that their exclusion would not profoundly affect our analyses. Review of all laboratory, demographic, and medical history data was approved by our institutional review board.

Primary exposure

'Variant hemoglobin' represented our primary exposure, defined as any hemoglobin phenotype containing a variant from normal adult hemoglobin (HbAA). All hemoglobin phenotyping studies were performed in June 2008 at a single laboratory site (LabCorp) using high-pressure liquid chromatography. Sickle trait was defined by high-pressure liquid chromatography as the presence of hemoglobin A and S (HbAS), and hemoglobin C trait was defined as HbAC. Patterns demonstrating elevated proportions of hemoglobin A2 and hemoglobin F relative to hemoglobin A identified those with β-thalassemia trait. We assessed the association of any variant hemoglobin with the outcome, as well as each identified phenotype individually.

Outcome

All patients were treated with the same intravenous ESA dosing protocol to goal Hgb $\geqslant 11.1$ <12.1 mg/dl with percent dosing adjustments made on the basis of measured hemoglobin level (Supplementary Figure S1 online). We defined higher-dose ESA, our primary outcome, as a dose of ESA equal to or exceeding 6500 U/treatment, equivalent to 19,500 U/week as all patients were prescribed three treatments weekly. This value was chosen based upon the distribution of ESA dosing in our study population, as well as the dose above which mortality increased in both USRDS data and secondary analysis of the CHOIR study.[24,40] Delivered erythropoietin doses were obtained as a summary measurement over the specified time period. For each patient, we calculated the per-treatment dose by dividing the total delivered dose by the number of treatments received over the 3-month period. For the purposes of sensitivity analysis, we also modeled alternative definitions of higher-dose ESA using other cutoff points of ESA dose per treatment, as well as a weight-based per-treatment definition for higher-dose ESA. For this latter analysis, higher-dose ESA was defined as a dose $>=100$ U/kg/treatment, representing greater than the 75th percentile in the study population.

Covariates

Demographics and medical history maintained in administrative databases at each dialysis unit were reviewed to extract information on our additional covariates including age, sex, body mass index, diabetes mellitus, history of GI bleed, tobacco abuse, and alcohol abuse. Diabetes mellitus was defined either as the ascribed cause of ESRD or as a comorbidity. Tobacco and alcohol abuse history were recorded as dichotomous variables indicating presence or absence. GI bleed was indicated as history of bleeding from any GI source. Vintage of dialysis was calculated by subtracting the age of onset of ESRD from the final date of the study. Iron dose per treatment was calculated in a similar manner to erythropoietin per treatment. All available laboratory studies were reviewed and averaged during the 3-month period for ferritin, iron saturation, parathyroid hormone, serum albumin, serum phosphorus, and adequacy studies (Kt/V). Interdialytic weight gain was taken as the difference between post-weight and pre-weight. Information regarding type of vascular access was extracted by review of administrative data sets maintained by the vascular access program. The type of access was coded as arteriovenous fistula, arteriovenous graft, or indwelling hemodialysis catheter. In the event a catheter was used at any time in the study period, patients were assigned to 'catheter' as their form of access for analysis purposes as this has been reported to be the most inflammatory of vascular access types.[43] We also determined the number of missed treatments, constructing a variable for a proportion of missed treatments by dividing by the total of prescribed treatments. We similarly evaluated days hospitalized over the treatment period, producing a variable for days hospitalized per treatment prescribed. Because hospitalization days and missed treatments were felt to represent redundant measurements ($\sigma = 0.6$, $P<0.0001$), we chose to adjust for missed treatments as this variable captured both non-adherence and hospitalizations.

Statistical analyses

Following evaluation of skewness and kurtosis, we calculated means for normally distributed continuous variables and report medians and interquartile ranges for continuous variables not normally distributed. Counts and percentages were determined for all categorical variables.

Prevalence ORs were obtained using univariate and multivariate logistic regression. Logistic regression was also used to assess the relationship between hemoglobin phenotype and higher-dose ESA.

The association of any variant hemoglobin with the outcome was assessed as a group and within each identified phenotype.

Because higher-dose ESA, as defined in our study, was not a rare outcome (that is, $>10\%$), we also used Poisson regression modeling to determine more conservative effect estimates where incidence rate ratios serve as a less-inflated measure of risk than ORs.[44] Two-sided hypothesis testing with an *a priori* level of significance 0.05 was used for all statistical inferences.

Potential covariates for inclusion in our models were derived from previous studies of anemia in ESRD and potential mechanisms that may influence anemia response. Measures considered as confounders included age, sex, body mass index, diabetes mellitus, iron dosing, ferritin, iron saturation, intact PTH, albumin, phosphorus, interdialytic weight gain, vascular access type, history of smoking, alcohol abuse, and GI bleed. Hemoglobin levels themselves were not included in our covariate pool as we felt that this measurement lay upon the causal pathway of our exposure. Within the included population, missing data were present in fewer than 3% of observations and constructed models used complete case analyses. Following univariate analyses, we compared models with and without interaction terms via likelihood ratio tests to assess for effect measure modification. Using a conservative level of significance of 0.15 for these tests, we did not identify any relevant interaction terms.

Because of the relatively small number of participants, we attempted to create a model using a conservative number of covariates. From our univariate analyses, we identified confounders as those covariates that were either associated with the exposure or with the outcome among the unexposed. Potential weak confounders identified included dialysis vintage, percent of missed treatments, dialysis unit, and serum albumin, iron saturation, iron dose, vascular access type, and history of GI bleed. Hospitalization days were also noted as a potential weak confounder, but were not included in the model, as this information was felt to be included in the missed treatment data. In addition, we chose to evaluate serum ferritin, intact parathyroid hormone, iron saturation, and Kt/V in our full model as these factors had been noted in earlier studies to be associated with erythropoietin response.[45–47]

A fully adjusted model using all possible confounders was constructed; we then created a simplified model removing covariates, using backward elimination and change-in-estimate testing. Only those variables the removal of which increased or decreased the exposure estimate by $\geqslant 10\%$ were retained in the simplified model. Using the constructed logistic regression models, we repeated these analyses using Poisson regression with the same covariates. We also evaluated the two primary hemoglobin variants separately (HbAS vs. HbAC) in univariate analysis. For the purpose of sensitivity analyses, we used alternative definitions of higher-dose ESA including a per-kg dose of ESA, substituting these for our primary outcome in our fully adjusted logistic regression model. To comment upon the racial difference in ESA dosing in our chosen dialysis clinics, we also compared median dose of ESA for the African-American patients with 60 Caucasian in-center hemodialysis patients from the same four clinics. These patients represented all of the Caucasians receiving treatment at the time of hemoglobin phenotyping and who met the criteria of having missed fewer than 20 treatments over the study period. All were verified to have normal hemoglobin phenotypes. All statistical analyses were performed using Stata 10.1 (ref. 48).

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure 1. Erythropoiesis-stimulating agent (ESA) protocol. Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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